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Habitat selection, behavior, and natural history of the newly described leaf chameleon Brookesia micra (Reptilia, Squamata, Chamaeleontidae; Glaw, Köhler, Townsend & Vences, 2012) on Nosy Hara, Madagascar

Andrew Villeneuve
SIT Study Abroad

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Andrew Robert Villeneuve

Advisors : Louis-Philippe d'Arvisenet, Barry Ferguson

Academic Director: Jim Hansen

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Researcher with adult female *Brookesia micra*

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Permit

Authorization to travel to and study in Nosy Hara National Park from April 8 to April 29 2015 as a student was obtained and paid March 27, 2015, No. 233/15/DG/DGA/DOP/CEE.

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List of Abbreviations

ANOVA – Analysis of Variance

CITES - Convention on International Trade in Endangered Species

CPUE – Catch Per Unit Effort

IUCN – International Union for Conservation of Nature

MNP – Madagascar National Parks

MYA – Million Years Ago

SE - Standard Error

SEM – Standard Error of the Mean

SVL – Snout-Vent Length

WWF – World Wildlife Foundation

Glossary

Tsingy – Sharp limestone karstic rock formations, found in Ankarana, Montagne des Français, and Nosy Hara.

Fady – Local cultural taboos. Many exist on Nosy Hara

Abstract

The micro leaf chameleon genus *Brookesia* is exclusively endemic to Madagascar and represents some of the smallest reptiles in the world. The newly-described species *Brookesia micra* is exclusively endemic to the islet Nosy Hara in the north of Madagascar, and is the smallest described *Brookesia* species member. There exists no density, behavioral, or habitat preference studies on *B. micra*. Here, the researcher presents preliminary density, behavioral, habitat selection, and natural history information on *B. micra*. A total of 117 *B. micra* had data collected on them between April 10 and April 23, 2015. Density was found to be 1950 *B. micra/*ha (assuming 70% detection) and through aerial analysis it is estimated that the population size of *B. micra* is between 70,000-95,000 individuals on Nosy Hara. This study found no significance of SVL between adult males and females, but did find significance between male and female juveniles/sub-adult SVL. This study also found no significance in roost height between males and females of adult and juvenile/sub-adult age classes. *B. micra* was significantly closer to *tsingy* outcroppings compared to trees both during the day and at night. In addition, higher abundance was positively correlated with slope, and a leaf litter-*tsingy* ratio of 1:1. However, *B. micra* was also found in abundance in habitats with little to no leaf litter, indicating that this is a *tsingy*-associated species. Defensive behavior such as perch release, dead leaf mimicry, and vibration were observed, as well as low movement between successive roost locations, a preference for slope, horizontal to head-up roost orientation preference, and a preference for dead twigs with small diameter as roost locations. The researcher supports the IUCN designation of near-threatened due to the high density and large estimated population size, but threats due to tourism and small range size could pose problems for the specie's conservation.

Introduction

Reptiles are one of the most diverse and endemic phyla of Madagascar's fauna, and are at high risk due to habitat degradation and climactic shifts – up to 39% of species are at risk of extinction (Jenkins et al. 2014). Miniaturization and microendemism are two features of many of Madagsacar reptiles as well as other fauna such as lemurs and amphibians (Glaw et al. 2012, Townsend et al. 2009, Wilmé et al. 2006). Microendemism on Madagascar proper has been theorized to be the result of watershed retreat during the Quaternary period (Wilmé et al. 2006), with subsequent effects of low vagility, genetic isolation, low gene flow, and small isolated land area which have been linked with miniaturization (Gaston 1996, Glaw et al. 2012). The micro leaf chameleon genus Brookesia displays extremes within the realm of microendemism and miniaturization, with 50% of species in this large genus coming from single type localities and exhibiting patterns of miniaturization in proportion with range (Carpenter and Robson 2005, Glaw et al. 2012, Townsend et al. 2011). As a result, many species of Brookesia are listed as threatened or endangered under CITES due to small ranges, preference for undisturbed habitat, and extremely specialized niche-filling (Carpenter and Robson 2005, Glaw et al. 2012). Brookesia miniaturization is a unique trait in Madagascar chameleons, mirrored by the dwarf African chameleon genus *Rhampholeon* and *Rippeleon* (Glaw et al. 2012), and most likely developed to fulfill extremely specific niches in small habitat ranges (Gaston 1996).

The *Brookesia minima* group is currently constituted of 11 different species found exclusively in the extreme north of Madagascar (Glaw et al. 2012) and are the only group of *Brookesia* to be found from both sides of the Tsaratanana mountains, as other *Brookesia* groups are found in small isolated rainforest pockets on either side (Raxworthy and Nussbaum 1995). They contain some of the smallest species of all *Brookesia* and the most cryptic (Glaw et al. 2012). Recently, *B. minima* was split up into several different species (*B. minima, B.confidens, B. desperata, B. tristis,* and *B. micra*) which each have their own extremely specific habitats on the basis of nuclear and mitochondrial DNA differences, and

differences in genital and physical morphology (Glaw et al. 2012). These splits are quite deep, being of Eocene to Miocene origin, with a minimum divergence in the *ND2* sequence of 18% in the sister taxa *B. desperata* and *B. tristis* (Glaw et al. 2012), all indicating long-term isolation of populations. In addition to *B. tuberculata*, they form what Glaw et al. (2012) have termed Clade A *Brookesia*, belonging to the extreme north of Madagascar. *Brookesia minima* group species all share characteristic extreme endemism to specific niches, although varying between tropical rainforest to dry deciduous *tsingy* forest (Glaw et al. 2012).

B. micra (Glaw, Köhler, Townsend, and Vences 2012) is a recently described member of the *B. minima* group exclusively endemic to the small limestone karstic ("*tsingy*") island of Nosy Hara. With an adult female SVL of 18.7-19.9mm and male adult SVL of 15.1-15.3mm, *B. micra* qualifies as the smallest described chameleon and lizard in the world, and qualifies to be on the list of the world's smallest amniotes (ranking dependent on type of measurement used, Glaw et al. 2012). *B. micra* is sister taxa to *B. minima, B. tuberculata, B. desperata,* and *B. tristis* with a divergence date of approximately 22.5 MYA (Glaw et al. 2012). Range could have extended further, but as of yet *B. micra* has not been found on the mainland or surrounding islets in the Nosy Hara archipelago, and so barring local extinction events and dramatic range shifts, Nosy Hara proper is and has been the only known habitat of *B. micra*. Glacial maxima during the Miocene could have allowed for a land bridge between Nosy Hara and the adjacent mainland when forest was more extensive in Northern Madagascar for *B. micra* ancestor to colonize, and with subsequent glacial minima and changing climate forced *B. micra* ancestor to adapt to a drier habitat, perhaps by engaging in miniaturization (Raxworthy and Nussbaum 1995). Glaw et al. (2012) question whether this could be an example of island dwarfism. Rafting from the mainland during a glacial minima could also have provided a colonization vector for *B. micra* onto Nosy Hara.

Because of *B. micra*'s small range and unknown population, it has earned an IUCN designation of Near Threatened (Glaw and Jenkins 2014). It has only been described in literature from two locations in a temporary dry river valley in the south of Nosy Hara (Glaw et al. 2012), and no reports on behavior, habitat preference, or density have been published. Anecdotal reports of rats brought on-island by fishermen (MNP 2010) and the possibility for total regime-shift due to small land area are possible conservation problems. A previous herpetological survey carried out in 2000 (Metcalf et al. 2007) within the archipelago and on Nosy Hara failed to detect *B. micra*, probably due to the extreme cryptic nature and small size of *B. micra* or the absence of used trails on the interior of the island. Trail development within Nosy Hara only began when it became a National Park in 2007. *B. micra* was discovered in litter and *tsingy* mosaic forest exclusively in the southern temporary stream valley on Nosy Hara, and was collected and observed either active in the litter or roosting in bushes (Glaw et al. 2012, Glaw and Vences 2007). Little natural history or habitat selectivity is known about *B. micra*, but generalizations can be made from the natural history of more well-known mainland *Brookesia* species. Most *Brookesia* chameleons forage during the day on the forest floor searching for ants and other small invertebrates, and retreat to roosting locations no more than a meter high up in vegetation at night, possibly for predator avoidance (Randrianatoandro et al. 2007, Razafimahatratra et al. 2008). 70-80% of *B. decaryi* choose to roost in trees, and usually have a head-up orienatation, with 70% choosing sprigs and 30% trunks (Razafimahatratra et al. 2008). Sex is believed to not affect roost height (Randrianantoandro et al. 2007), but adults are shown to prefer higher roost sites than juveniles in *B. decaryi* (Razafimahatratra et al. 2008). *Brookesia* become pale at night while roosting, allowing for easy detection, but are brown during the day (Raxworthy 1991). Glaw et al. (2012) reported that *B. micra* was also spotted with white head and back coloration, possibly as a stress response. Many are thought to prefer gallery or riparian forests with plenty of leaf litter and are very intolerant to habitat modification (Randrianatoandro et al.

2008). Perch release, dead leaf imitation, and escape have been observed in *B. minima* and other members of the *Brookesia* genus as defensive features (Raxworthy 1991).

The purpose of this project is to document as much as possible the habitat, spatial preferences, and behavior of *B. micra* on Nosy Hara as well as obtaining initial density and abundance estimates and to extend their known range on the island. Modified survey techniques after Jenkins et al. (2003), Jenkins et al. (1999), Lowin (2012), and Razafimahatratra et al. (2008) will be used. Intensive transects and opportunistic searching in known *B. micra* range will be used to quantify abundance, density, behavior, morphological characteristics, and microhabitat preferences, while quadrats will be used in the same known range to understand habitat changes within the valley. Exploratory searches on other parts of the island will also be used to extend range on the island.

Methods

Study Location

Nosy Hara (12° 14.945' S 49° 00.455' E) (Figure 1.) is a limestone karstic island (termed "*tsingy*" in Malagasy) of about 270 ha lying around 6km offshore of the northwest coast of Madagsacar in the Baie du Courrier, and is located in the Diana Province, Antisiranana Region. The island is surrounded by water at least 25m deep (Glaw et al. 2012), and is located within the new Nosy Hara National Park which came into being in 2007 through the work of MNP (Madagascar National Parks) and WWF (World Wildlife Fund). The island is dominated by xerophytic vegetation on the higher, more exposed limestone massifs and dry deciduous forest on valley floors. The deciduous valley forest was lightly selectively logged in the past before the creation of the park, and so while many large softwood trees (like fig) grow in the southern valley, the forest is in a late secondary growth phase. It should be noted, however, that multiple *fadys* (taboos) exist on the island and the entire archipelago is viewed as sacred to the

Sakalava-Antankarana people who live on the adjacent mainland (MNP 2010). Data was collected in the early dry season between April 10 and April 23, 2015.

There exists no permanent fresh water on the island, but a dry stream bed that holds water during the rainy season exists in the south, where it drains the southern bowl-like valley onto the southern beach. A trail, called *Brookesia micra* Trail in honor of the discovery of the chameleon, runs the length of the streambed before mounting the eastern limestone massifs and on which every 50m are marked.

This trail was used as the central transect line for a majority of transects and quadrats obtained in this project. In addition, there exists the Chauve-Souris trail which mounts an isolated *tsingy* outcrop in the middle of the southern valley and exploratory searches for *B. micra* were undertaken here. Finally, a third path covers the entire northern length of the island over *tsingy* massifs and into forested valleys, and in one of these valleys a second exploratory search for *B. micra* was undertaken.

A concrete path and stairway was laid down on the southern streambed between meter marks 200m and 300m and between 450m and 500m by pas MNP administrations to abet tourism on the island (Figure 2). This path covers 21% of the streambed, which is currently the only known habitat of the exclusively endemic *Stumpffia hara* and *Mantella cf. viridis* (Crottini et al. 2012, Köhler et al. 2010, Glaw pers. comm. 2015). The walkway destroyed many breeding pools for these frogs and sped up water flow into eroded channels along the path. The effects of the walkway are unstudied on *B. micra*, and are likely significant if the population is restricted to the riparian zone of the streambed.

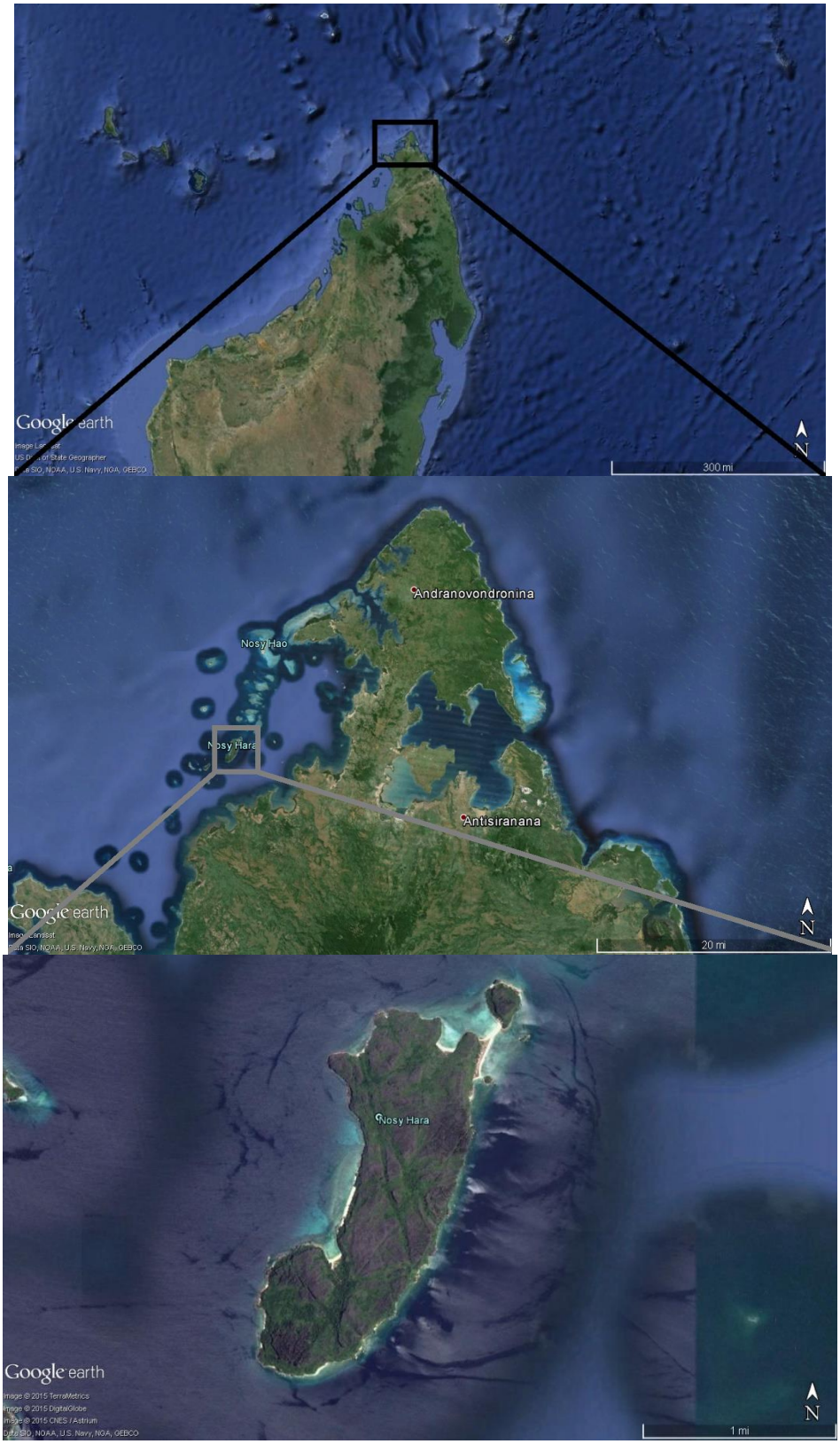

Figure 1. Map of Nosy Hara where this study was carried out with its location within Madagascar.

Figure 2. Concrete stairwell built by MNP to facilitate tourism. Image at left is the first half of the lower stairway between meter marks 200m and 300m, the image to the right up is the entire second stairway between 450m and 500m. Both are built on the bed of the temporary stream used by *Mantella cf. viridis* and *Stumpffia hara* as well as being extremely close to *B. micra* habitat.

Transects

Diurnal and nocturnal transects allowed the researcher to gather general density, morphological, gender and size class, microhabitat and spatial behavior data. Detection probabilities in reptiles are almost always <1, and have wide variance between species (Durso et al. 2011, McDiarmid 2012). Therefore, density estimates will take into account an estimated detection probability determined by the researcher. Every 50m along the central path, a 5m transect line was randomly laid down perpendicular to the trail at least 1m into the forest to eliminate edge effects and ecotone changes (Jenkins et al. 1999, Lowin 2012), and left for 24 hours before data was taken to ensure minimal disturbance to existing *B. micra* on transect. (Lowin 2012). Transects were then carefully crawled by a single researcher on hands and knees, searching 30cm of either side of the line (60cm total) as intensively as possible leaf litter during the day and low-lying twigs at night (Jenkins et al. 1999). Crawl speed varied with vegetation density, and usually ranged from 4-5 min/m. Therefore, crawl speed was determined more by approaching 100% detection than by a uniform crawl speed (Jenkins et al. 1999), but never exceeded more than 40min and was thus chronologically normalized. GPS coordinates and

altitude were recorded for each transect line. Every *B. micra* detected on the transect line was sexed, males having a larger tail base due to inverted hemipene compared to females (Glaw et al. 2012), and had its SVL measured to the nearest 1mm with a plastic ruler. Individuals were sorted by age class, with adults having a SVL≥14mm and juveniles/sub-adults SVL≤13mm (Glaw et al. 2012). Adult *B. micra* often have orange colored tails while juveniles do not (Glaw et al. 2012) In addition, height above or below leaf litter, canopy cover, and declination were recorded for each *B. micra* as well as its distance along the transect and distance from the riparian zone. Canopy cover was recorded by establishing a 5x5 grid on a clear CD case which was then held 1m above the ground and visually inspected for the amount of squares with more than 50% sky. Slope was recorded using a protractor with a plumb line with the 90° mark set perpendicular to the ground. Microhabitat data for each *B. micra* was also taken by measuring the distance each chameleon was from the nearest *tsingy* outcropping (emergent from litter), the nearest tree distance, and the diameter at 10cm height. Notes on substrate and roosting orientation were further recorded to establish patterns. Transects were crawled once at night, moved no more than 5m up or down the path, and then re-laid for a future night transect. All night transects took place between 17:00 and 21:00. Almost all transects were laid on the left side of the trail with the exception of the 700m transect which was laid to the right due to a shear *tsingy* cliff. During the day, the researcher carefully turned over leaf litter abutting *tsingy* outcroppings and trees with a stick, and at night a headlamp was used to find *B. micra* roosting in low vegetation since most Brookesia becomes very pale when roosting (Raxworthy 1991). All disturbed litter was carefully pushed back into place after searching, and the researcher was careful to step on *tsingy* or in already searched litter to reduce the chances of habitat degradation.

Opportunistic Searches

Diurnal and nocturnal opportunistic searches allowed the researcher to increase the sample size for morphological, gender and size class distribution, and microhabitat data. While the researcher was crawling transect lines, one to two trained guides searched for *B. micra* anywhere between the current 50 meter mark and the next 50 meter mark. Guides would then inform the researcher of the location of each *B. micra* so that the researcher could take measurements to lessen recorder bias. Guides were careful to follow similar habitat conservation techniques listed above, and never searched more than 10m from the trail. Searches never lasted more than 40min per each 50m, so all abundance data is chronologically normalized.

Habitat Quadrats

Diurnal habitat quadrats allowed the researcher to gain a clearer picture of habitat shifts each 50m along the trail. As opposed to the transects, the quadrats were selectively placed in locations the researcher deemed most representative of the habitat between the meter marks. Quadrats were 2m by 2m ($A=4m^2$) to maximize representative sampling which transects sometimes failed to do. In each quadrat, percent coverage of *tsingy* and litter were recorded, as well as canopy density, slope, litter composition, number of trees and their size classes (Large having a diameter at 10cm height more than 10cm and small having a diameter at 10cm height less than 10cm). Litter composition was recorded on a scale of 1 to 4, with 1 constituting poor to no litter cover of small leaves or sticks and poor or sandy soil and 4 constituting rich and deep litter with large leaves and moist soil.

Geographic Data

Geographic data was recorded using a Garmin eTrex Vista HCx GPS with accuracy always at least ±5m and altitude data calibrated to sea level. Waypoints and altitude information were taken at each 50m

interval on the southern trail and any *B. micra* discovered during exploration had individual waypoints recorded. Potential habitats that weren't searched also had waypoints recorded.

Exploration

Exploration was undertaken in two locations on the island to search for new potential and real *B. micra* habitat. This included the Chauve-Souris path, located roughly at 12° 15.124' S 049° 00.389' E and wooded valleys on the northern *tsingy* path, especially between the coordinates 12° 14.533' S 049° 00.583' E, 12° 14.624' S 049° 00.549' E, 12° 14.360' S 049° 00.659' E - 12° 14.347' S 049° 00.629' E, and 12° 14.115' S 049° 00.658' E - 12° 14.354' S 049° 00.714' E. Searches lasted only 2 hours at most. Any *B. micra* found had SVL, gender, age class, and altitude data recorded.

Analysis

Geographic information was processed using Google Earth Pro v. 7.1.2.2041 . Statistical analysis was completed on Graphpad Prism v. 6.05. Two-way ANOVAs were used to analyze SVL by sex and age class, to analyze roost height by sex and age class, and to analyze tree and *tsingy* proximity preferences at night and during the day. The author justifies the use of two-way ANOVA to analyze the above data because he desires to known the response to two factors and if there exists any interaction between factors (GraphPad Statistics Guide 2015, McDonald 2014). Normality and equal standard deviations are also assumed in all data sets (McDonald 2014). Bonferroni post-hoc test was used to correct for multiple comparisons because of its more conservative nature compared to the Holm-Šidak test and because of the low number of comparisons to be analyzed (GraphPad Statistics Guide 2015). The Bonferroni test was used assuming independence between individual tests (McDonald 2014). Standard Error of the Mean (SEM) was used to show precision of the mean, and the confidence interval was set at 95% (GraphPad Statistics Guide 2015). The level of statistical significance was set at p=0.05 (Zar 1984).

Results

Gender/Size Class Breakdown

In all, 117 *B. micra* were found and had some type of data collected on them, with 74 found at night and 43 during the day. Along the path, CPUE (Catch Per Unit Effort) rate was 0.85 *B. micra*/hr and most *B. micra* were found at three abundance peaks around 250m, 450m-500m, and 600m-650m (Figure 3). 25 adult females, 22 juvenile and sub-adult females, 16 adult males, and 43 juvenile and sub-adult males were found. Two-way ANOVA analysis rejected the null hypothesis that there was no difference between sex and age class SVL (two-way ANOVA, $F_{1,102}$= 7.7, interaction p = 0.0064, n=106). However, post-hoc Bonferroni's multiple comparisons showed that juvenile/sub-adult males were significantly larger than juvenile/sub-adult females and that adult females were not significantly larger than adult males. Adult female B. micra had a mean SVL of 17±0.40mm ($\bar{x}$±SEM), juvenile and sub-adult females had a mean SVL of 11± 0.30mm, adult males had a mean SVL of 16±0.70mm, and juvenile and sub-adult males had a mean SVL of 12±0.18mm.

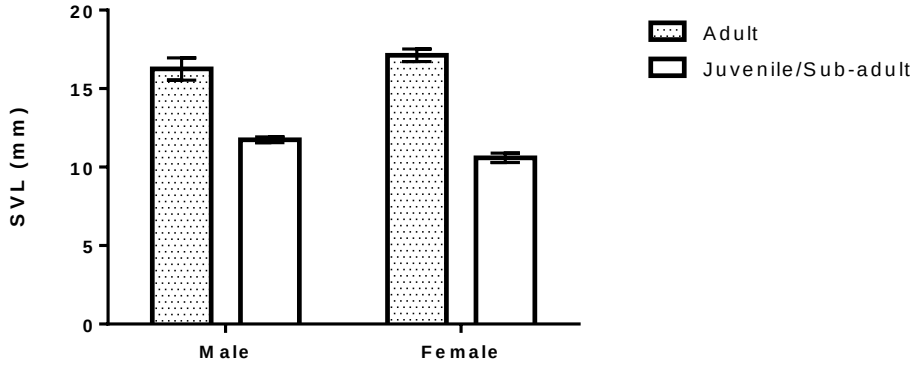

Figure 3. SVL of female and male *B. micra* in adults (SVL≥14mm) and juveniles/sub-adults (SVL≤13mm). We were able to reject th null hypothesis of no interactions between males and females and age classes (two-way ANOVA, $F_{1,102}$= 7.7, interaction p=0.0064, n=106). Post-hoc Bonferroni's multiple comparison tests showed that there was significance between male and female juvenile/sub-adults but no significance between male and female adults. Error bars represent standard error of the mean (SEM).

Density

Density was calculated from both day and night transects. Area surveyed was $42m^2$ for each type of transect for a total $84m^2$. 3 *B. micra* were found on day transects for a raw density of 0.071 *B. micra*/m^2, and 9 were found on night transects for a raw density of 0.21 *B. micra*/m^2. Assuming the area surveyed to be an almost linear strip 700m long by 20m wide for 1.4 hectares and that the night transect density to be the more accurate density approximation, estimated *B. micra* population in area surveyed is 2100 individuals and a density of 1500 *B. micra*/ha. While the researcher constructed the transects to approach 100% detection probability of *B. micra*, the researcher estimates a more accurate night detection rate of 70%. Therefore, the adjusted population is more likely close to 2730 individuals in the area surveyed with a density of 1950 *B. micra/*ha. Since Nosy Hara is not completely constituted of ideal *B. micra* habitat, Google Earth Pro v.7.1.2.2041 was used to calculate the area of forest which aerially appeared to be closest to the forest where *B. micra* was studied in the southern valley. Roughly 48.9 hectares (roughly 20% of the island's area) appear to be suitable habitat, and assuming equal *B. micra* densities throughout Nosy Hara and an adjusted night density, the total population could be between 70,000-95,000 individuals (range indicates raw and detection-rate adjusted densities).

Microhabitat and Behavior

B. micra showed a significant trend to be in closer proximity to *tsingy* outcroppings than to trees during diurnal and nocturnal transects (Figure 4). Two-way ANOVA analysis rejected the null hypothesis that there was no significant difference for *B. micra* distance from *tsingy* or trees (Two-way ANOVA, $F_{1,198}$=6.6, interaction p=0.011, n=101), and post-hoc Bonferroni tests showed that *B. micra* was found significantly closer to *tsingy* than to trees during both day and night treatments. In addition, *B. micra* was found to be significantly father away from trees at night than during the day, but that there was no

significance in distance of *B. micra* from *tsingy* during day and night treatments. Distance from trees during the day was 44±9.6cm ($\bar{x}$±SEM) and at night was 78±7.1cm. Distance from *tsingy* during the day was 11±4.0cm and at night was 9.7±2.0cm.

Nocturnal transects revealed no significance in *B. micra* roost height, regardless of sex or age class (Figure 5.). Two-way ANOVA analysis revealed that the null-hypothesis could not be rejected (Two-way ANOVA, $F_{1,63}$=1.9, p=0.17, n=67). Further post-hoc Bonferroni tests revealed no significance in any age class or sex comparison of roost height in *B. micra*. Adult female B. micra had a mean roost height of 7.7±2.4cm ($\bar{x}$±SEM), juvenile/sub-adult females had a mean roost height of 2.6± 0.58cm, adult males had a mean roost height of 5.8±1.4cm, and juvenile/sub-adult males had a mean roost height of 4.6± 0.81cm. Mean roost height regardless of age class or sex was 5.0±0.67cm.

Opportunistic observations indicated that *B. micra* preferred to be on the down slope when abutting against a *tsingy* face or tree root on a slope. This preference was not integrated into the experimental design.

Roost orientation of *B. micra* varied widely, with the majority of chameleons preferring to be on dead twigs parallel to the ground, but many adopted a head up orientation on twigs with steeper slopes. However, several cases of head-down behavior were observed in roosting *B. micra*, and even one instance of a completely inverted, head-down orientation. All *B. micra* chose extremely small diameter dead twigs (less than 5mm) as roost sites.

Several times over the transect the author noted that several *B. micra* were found within 1 or less meters from each other. This included three possible mated pairs (Figure 3). One of these pairs was marked and observed over five consecutive nights. The female returned every night within 15cm of the same roost perch, and the male was observed three of these nights non-consecutively. The female was

observed climbing slowly along these roost perches as late as 6:00 and as early as 17:00, in addition to the normal roost observing times between 19:00 and 21:00.

Novel defensive mechanisms were recorded in *B. micra*, above all in roosting individuals. Individuals found during the day tended to actively move in response to litter disturbance. However, at night, several defensive mechanisms were recorded, including perch release and “dead leaf” imitation behavior. “Dead leaf” imitation behavior constituted of rolling onto the dorsal side and tucking of the limbs to imitate a small dead leaf. Three individuals, two adults and one juvenile, also displayed vibration defense, wherein the researcher felt intercostal muscle vibration when the individuals were gently held between thumb and forefinger. In addition, almost all roosting *B. micra* had the lighter gray coloration associated with roosting and sleeping and almost all displayed typical daytime brown coloration. When disturbed when awake during the day or at night, all *B. micra* displayed typical stress coloration consisting of a darkening of the body and the presence of a light gray stripe running from the top of the dead along the dorsal ridge (Figure 6.).

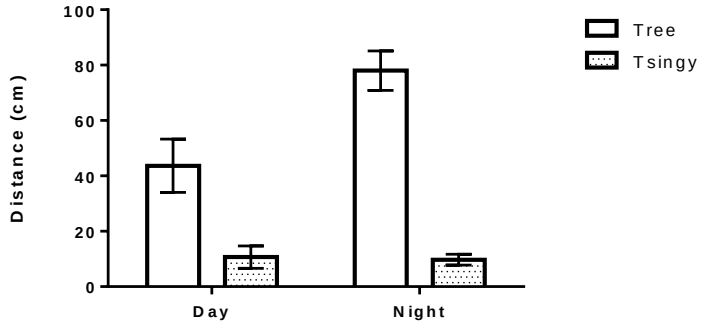

Figure 4. Distance of *B. micra* from the nearest tsingy and nearest tree during diurnal and nocturnal transects and opportunistic searches. We were able to reject the null hypothesis of no significance of *B. micra* distance over treatments (Two-way ANOVA, $F_{1,198}$=6.6, interaction p=0.011, n=101). Post-hoc Bonferroni's multiple comparison test showed significance between tree distance day and night but no significance between tsingy distance between day and night. Error bars represent standard error of the mean (SEM)

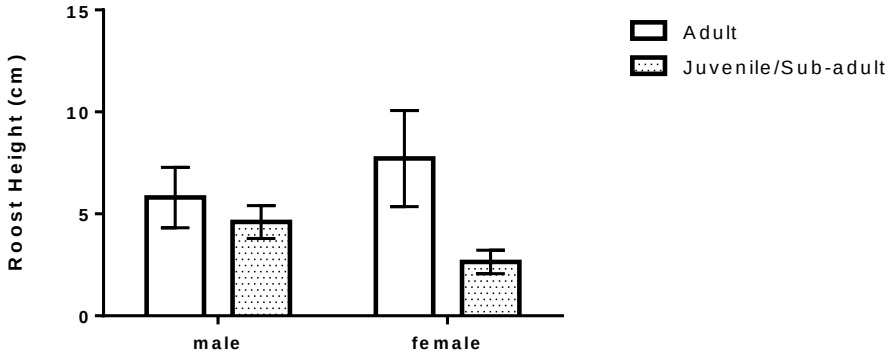

Figure 5. Nocturnal vertical roost heights of *B. micra* classed by gender and age class, by adults (SVL≥14mm) and juveniles/sub-adults (SVL≤13mm). We were not able to reject the null hypothesis of no interaction between male and female and age classes (Two-way ANOVA, $F_{1,63}$=1.9, interaction p=0.17, n=67). Post-hoc Bonferroni's multiple comparison tests revealed no significance in any category. Error bars represent standard error of the mean (SEM).

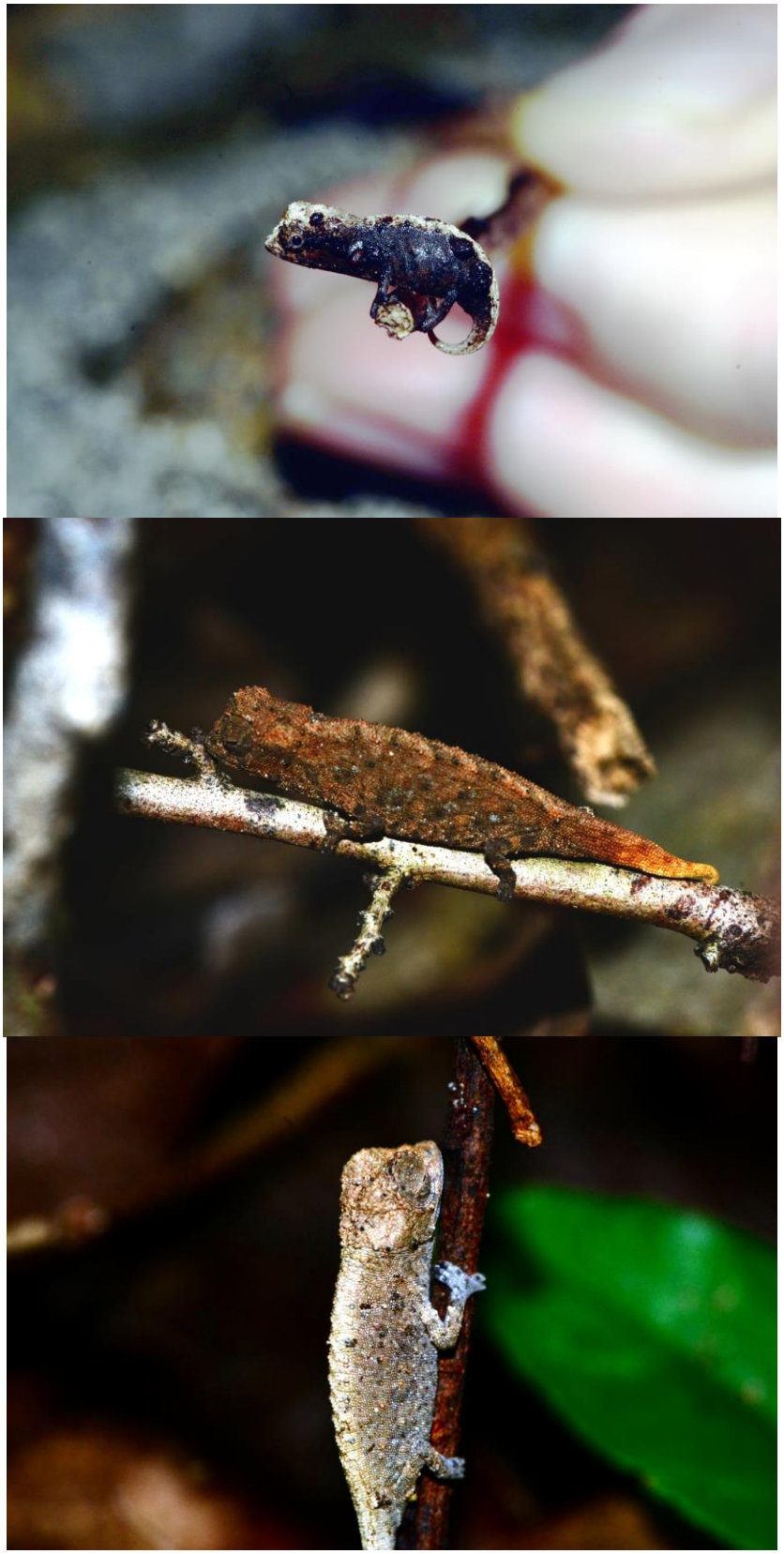

Figure 6. Representative *B. micra*. Top, juvenile displaying stress coloration. Middle, adult female displaying daytime awake coloration. Bottom, sub-adult male displaying roost coloration.

Habitat and Range

Habitat quadrats and GPS/altitude (Figure 7., Figure 8.) data were used in conjunction with *B. micra* abundance to determine ideal habitat and to determine the range of *B. micra* on Nosy Hara. 113 *B. micra* were observed on the southern trail, with a CPUE of 0.85 *B. micra*/hour. Most *B. micra* were found between 450m and 500m, but other abundance peaks existed between 200m and 300m and 600m and 650m. There was a noticeable decrease in abundance on the flatter sections of the trail (Figure 9). The first 50m of the trail had very little *tsingy*, leaf litter, and poor leaf litter quality. These two meter marks were on sandy soils bordering a small mangrove system and represented transition between dune and dry deciduous forest. The dry deciduous forest begins soon before the 200m mark with an elevation increase and this marks the first spike in *B. micra* abundance. Leaf litter quality and percent coverage increases around 150mand doesn't drop of until the 550m mark, while percent *tsingy* cover has two spikes, one around 150m to 200m and one from 550m to 700m. *B. micra* abundance appears to peak when *tsingy* and leaf litter percent cover are equal – around 50% each, at 200m-250m and more weakly around 450m-550m. *B. micra* has a lower abundance when *tsingy* percent cover is lower than percent cover of litter. Overall, *B. micra* appears to be in habitats with at least 20% *tsingy* coverage, and generally prefers high quality leaf litter (at least a litter composition value of 3, Figure 10), with the exception of around the 700m mark which was heavily dominated by *tsingy* with a few sticks. *B. micra* found at this mark were actively crawling on the *tsingy* rock surface and in small litter pockets of *tsingy*. Images of each quadrat mark with abundance found at each meter mark can be found in Appendix A.

Exploratory *B. micra* searches beyond the southern valley trail only revealed *B. micra* at high (41m-43m) altitude on the Chauve-Souris trail (Figure 11). This trail mounts an isolated *tsingy* outcropping in the middle of the southern part of the island, and is quite far (at least 100m horizontal and 20m vertical) from the valley floor. 5 *B. micra*, 1 adult female, 2 juvenile females, and 2 juvenile males, were found

along a dry drainage with *tsingy* outcroppings in a dry deciduous forest. Further explorations to the north of the island at the coordinates listed in the Methods section revealed no *B. micra* during quick searches, but the habitat appeared to be of good quality (Figure 11).

Figure 7. Map of transect locations in southern valley (*Brookesia micra*) trail. Markers follow rough path outline.

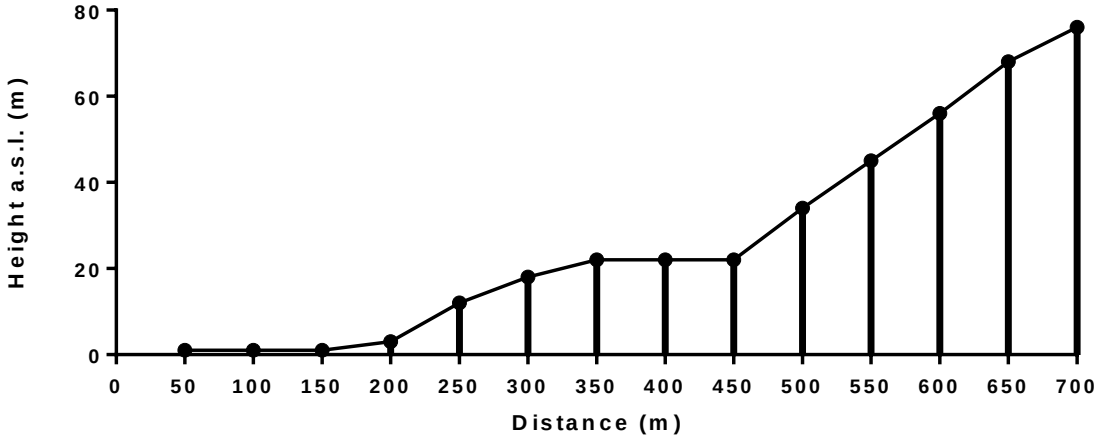

Figure 8. Altitude above sea level (a.s.l.) of the *Brookesia micra* path between 50m and 700m where the majority of data was recorded. Maximum altitude was 76m. Altitude was recorded using a Garmin eTrex Vista HCx GPS calibrated to sea level.

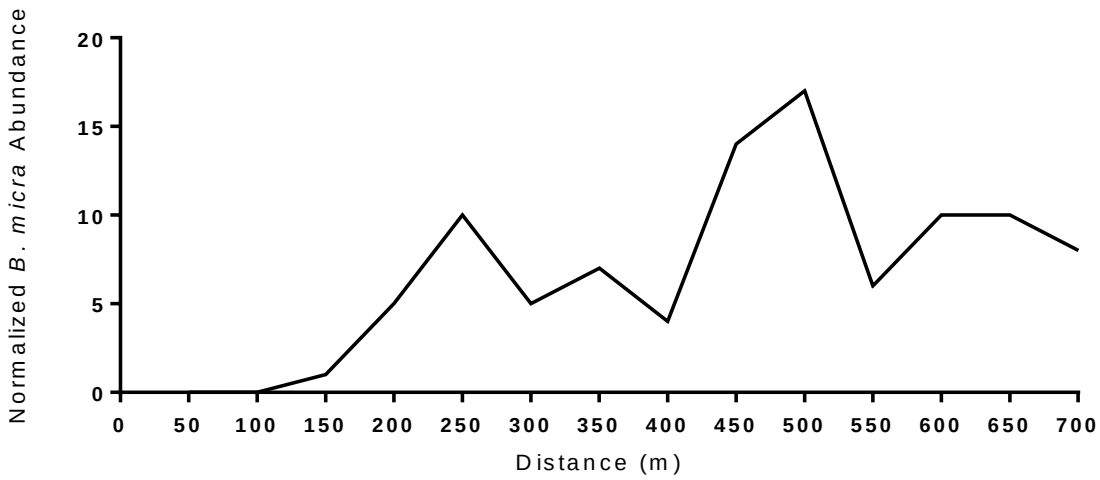

Figure 9. Total number of *B. micra* found, including diurnal and noctunral transects and opportunistic searching, along the 700m path. Results were naturally normalized, as transects were crawled at consistant rates of around 1m/4min to 1m/5min and opportunistic searches were limited to 30min. General trend was an increase of *B. micra* abundance around 450m-500m with abundance peaks at 250m and 600-650m. n=97, 113 search hours, CPUE=0.85 *B.micra*/hour.

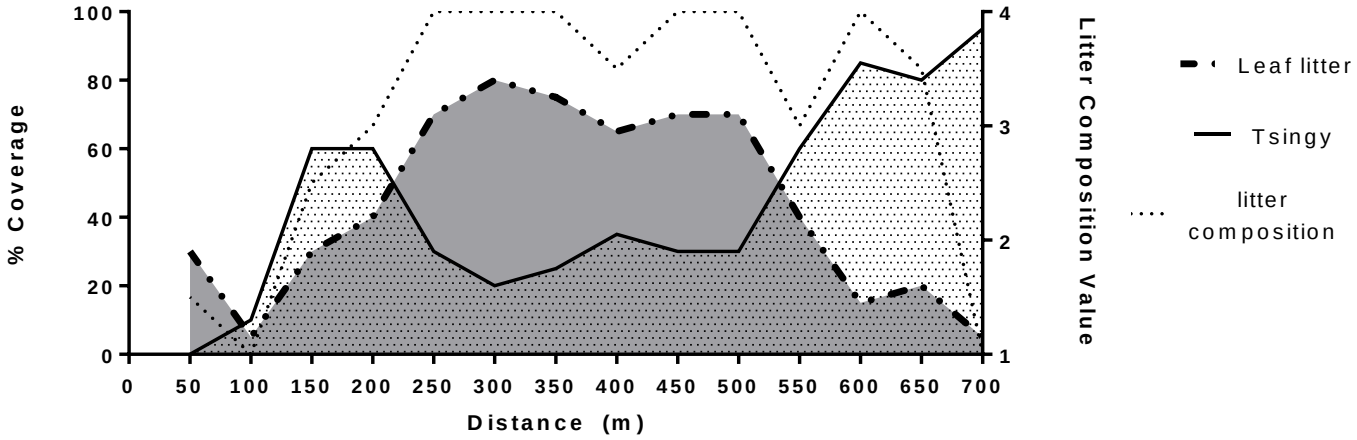

Figure 10. Visual percent coverage of tsingy and leaf litter estimates and leaf litter composition in 2mx2m quadrats (A=4m^2) placed at each 50m interval along the *Brookesia micra* path. Leaf litter composition values ranged from 1, which equaled little to no leaf litter litter with or without sticks, to 4 which equaled rich, deep litter with large dead vegetable matter. Leaf litter predominated quadrat composition between 250m and 500m, while tsingy dominated between 100m and 200m as well as from 500m to 700m. Litter composition was at its richest between 250m and 650m.

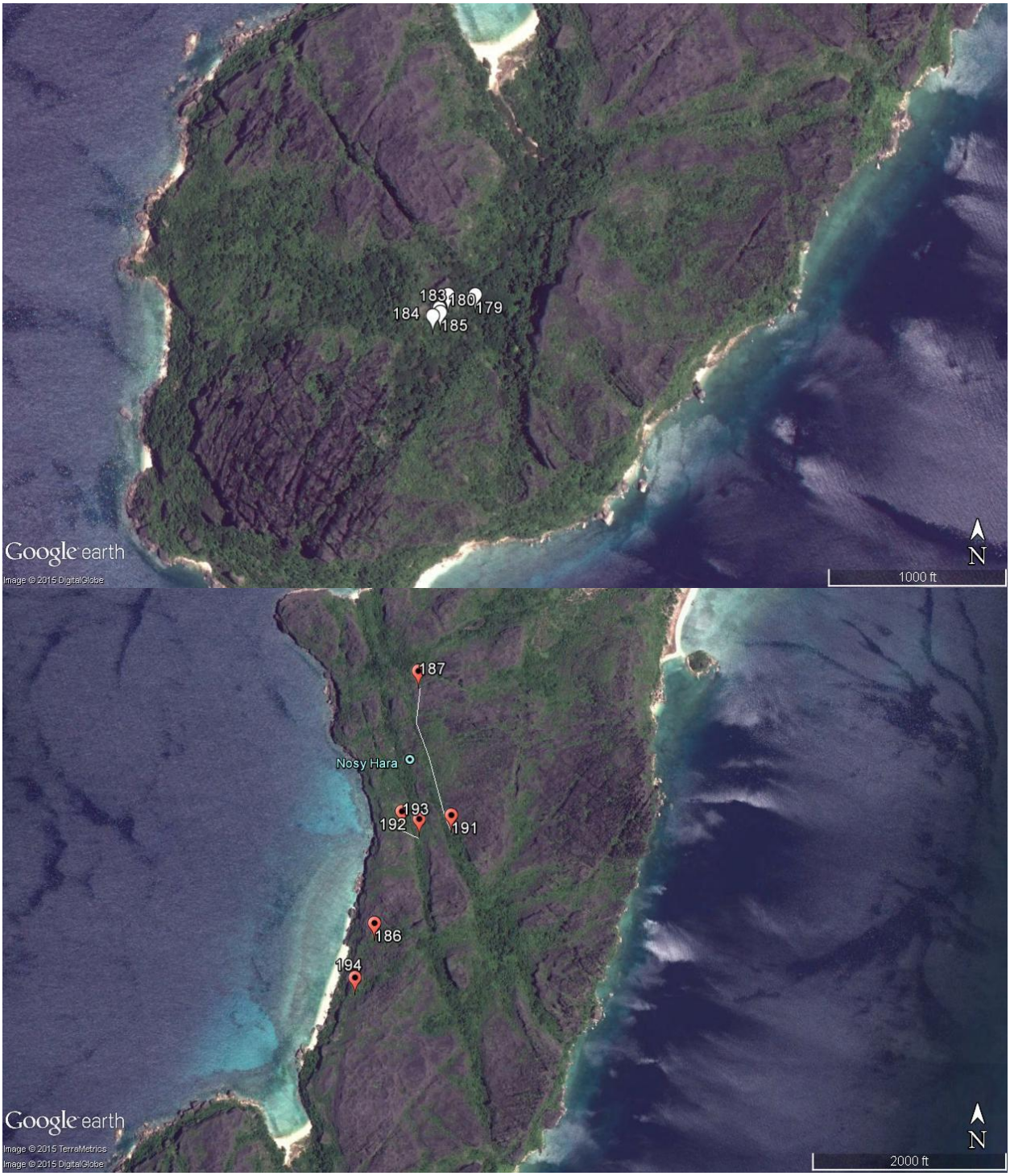

Figure 11. Map of exploratory searches. Top map indicates each *B. micra* found on the Chauve-Souris path. Bottom map indicates possible habitats for *B. micra* in the north of the island along the *tsingy* path. Lines between points indicate section of trail with good habitat, points indicate smaller areas with good habitat. Waypoint 194 was searched, with no found *B. micra* in 2 hours.

Discussion

Results obtained from this project suggest that *B. micra* has a wider range and habitat tolerance than previously believed, indicating that *B. micra* could be present elsewhere on the island as well as elsewhere in the archipelago or the mainland. In addition, many behavioral aspects common to the *Brookesia* genus have been confirmed to be present within *B. micra.*

This study presents a preliminary density based on 14 5m x 0.6m night transects in the *Brookesia micra* trail valley. Night transects are much more reliable indicators of *Brookesia* density due to the greater ease of finding them at night with a headlamp compared to leaf litter sifting during the day. 1950 *B. micra*/ha were calculated assuming 70% detection rate (1500 *B. micra*/ha raw density), which is a much higher density rate than previous studies of *Brookesia* density which found densities of 339.77±162.61 *B. stumpffi*/hectare (Density±SE) in Ankaz Forest, Montagne d'Ambre (Lowin 2012), 37.8±7.4 *B. nasus*/hectare (D±SE) in Ranomafana National Park (Jenkins et al. 1999), and 88.49 *B. thieli/*hectare and 20.69 *B. minima*/hectare in Andranomay Classfied Forest (Jenkins et al. 2003). Carpenter and Robson (2005) believe this to be because of census difficulties and overall low density of the *Brookesia* genus. This study, however, shows that *B. micra* can be found in incredibly high densities. Most likely, this is due to presence of good habitat all along the transect line, particularly in areas of high *B. micra* abundance. *B. micra* appears to benefit from extremely high densities to compensate for small range (documented 1.4 ha, theorized maximum 48.9 ha). Miniaturization by *B. micra* could therefore be an adaptation to allow for maximum population size in an area with minimal ideal habitat area. Miniaturized organisms often have reduced egg clutch sizes with larger individual eggs (Hanken and Wake 1993), as was reported by Glaw et al. (2012) in *B. desperata* and *B. tristis* with a clutch size of two eggs. This could allow more maternal energy investment for better hatchling survival, and thus a more constant growth rate – *Brookesia* could therefore classify as a *K*-selected organism.

Miniaturization of *B. micra* could also have arisen as a solution to microhabitat selection. *B. micra* strongly favored proximity to *tsingy*, which could provide cover from predators (including the coucal *Centropus toulou* anecdotally) or maintain moisture more than open areas or near tree trunks. The extremely high rugosity of *tsingy* provides many holes and crevices in which the miniaturized *B. micra* could hide during the heat of the day. *B. micra* miniaturization might therefore favor exploitation of more of these hiding places within *tsingy*. The significantly closer proximity of *B. micra* to trees during the day compared to night indicates that *B. micra* engages in day movement to hunt for food, and it is possible that areas closer to trees have a higher prey density than by *tsingy*. On days with bright sunlight, heat, or just after a rainstorm, *B. micra* were quite difficult to find. This *tsingy* also complicated the density/abundance data obtainment process, necessitating the night transects to obtain this data. *B. micra* abundance peaks appear to be correlated with *tsingy* and rich leaf litter, with *tsingy* percentage cover being higher than litter. Abundance tends to drop with lower *tsingy* percentage and flatter ground. *B. micra* abundance higher up on the trail (between 500m-700m) was characterized by habitats more and more dominated by *tsingy* and rapidly decreasing leaf litter composition and percent cover. This was extremely obvious at the 700m mark, which was dominated by *tsingy* boulders on a steep flank with almost no litter except for on the surface of *tsingy* in little pockets. It appears, therefore, that *B. micra* is highly associated with *tsingy* presence on sloped valley sides. Leaf litter appears to not have as much of an effect on *B. micra* abundance, despite offering moisture and other types of protection. *Tsingy* could offer better protection from predators and the elements, or just be the dominant feature of the habitat on Nosy Hara to which *B. micra* has adapted. Slope could also allow for more water shedding, and is also a more common habitat element on Nosy Hara compared to flat valley floor, which is only present for around 100m on the trail. Furthermore, the preference of *B. micra* to be on the downslope of *tsingy* indicates that they could provide protection from water running downslope. These characteristics make *B. micra* unique among *Brookesia* in that it does not appear to necessarily

associated with leaf litter or live plants like other *Brookesia* members from *tsingy* forests (*B. peramata, B. exarmata, B. brygooi,* and *B. bonsi* Randrianantoandro et al. 2008, Randrianantoandro et al. 2007). Therefore, the range of *B. micra* on Nosy Hara could extend elsewhere on the island where litter is minimal and poor in quality but where shaded *tsingy* boulders exist. However, it should be noted that even at the 700m mark where leaf litter was extremely rare, *B. micra* was observed at night on small twigs with no leaf litter matrix over bare *tsingy*. It can be concluded that *B. micra* can inhabit multiple types of habitat between dense dry deciduous forest to extremely rugose, dry *tsingy* rubble falls with no litter. This flexibility in habitat preference could therefore explain *B. micra*'s resilience in a changing landscape and small range size.

Roost height did not vary significantly between males and females regardless of age class. A study by Razafimahatratra et al. (2008) revealed higher adult roost heights in three sympatric *Brookesia*, but also confirms this study's finding of no significant difference between male and female roost height. It is interesting to note that *B. micra* had a lower mean roost height (5.0±0.67cm, $\bar{x}$±SEM) compared to the next lowest roost height in *B. exarmata* (roughly 20cm, Randriananatoandro et al. 2007). Therefore, the researcher assumes that either *B. micra* is a unique exception to age-class or sex determined roost height or there existed problems with sexing and classification of age. Problems with the SVL between adult males and females indicate that the latter could be the more likely problem, since Glaw et al. (2012) reported significant differences of adult female (SVL 18.7-19.9mm) and adult male SVL (15.1-15.3mm). However, the breeding patterns and lifespan of *B. micra* are unknown, and so it is possible that many *B. micra* have not reached maximum breeding size yet, since this study was completed in the beginning of the dry season and the Glaw et al. (2012) study in the late wet season. Future research should attempt to confirm or refute this paper's SVL and roost height distributions by sex.

Heads-up and horizontal roost orientation has been observed in many *Brookesia,* probably to increase water shedding during rain (Raxworthy 1991, Razafimahatratra et al. 2008). Observed orientation of *B. micra* closely matches these preferences. In addition, *B. micra* had an almost exclusive preference of roost location on dead twigs, whereas past research has mainly has observed *Brookesia* on live plants (Razafimahatratra et al. 2008). The preference for dead twigs despite availability of live plants in some areas could be an adaptation for a drier forest climate on Nosy Hara.

The very presence of *B. micra* as such a deeply genetically divergent species from its sister taxa on Nosy Hara is in itself a fascinating problem of dispersal and niche occupation. Nosy Hara is separated by 6km of 25m deep water in Baie du Courrier, and was likely connected to the mainland at some point in the Pleistocene Epoch during periods of glacial maxima (Miller et al. 2011). Base *tsingy* is of Jurassic limestone origin (Veress et al. 2008), and at the *Tsingy* de Bemaraha in west-central Madagascar, did not begin forming until around 1.8 MYA in the Pleistocene Epoch (Shea 2009), well after *B. micra* divergence in the Miocene Epoch 22.5 MYA (Glaw et al. 2012). However, sea levels were 25m-35m higher in the Pliocene 3 MYA than today (Rahmstorf 2007). Given the maximum altitude of *B. micra* in this study was 76m, available habitat was much smaller, although possibly more like mainland forest habitats before *tsingy* development. This means that either *B. micra* ancestor lived on Nosy Hara proper before it became separated from the mainland during a Pleistocene glacial minima, or that it rafted over to the island. In either case, *B. micra* most likely arrived on Nosy Hara when the habitat was more similar to a tropical humid forest, much like the current forest on Montagne d'Ambre. Wilmé et al. (2006) propose forest corridors like this could have existed all over Madagascar during climactic shifts between glacial maxima and minima. As soil eroded away and *tsingy* began to develop (Veress et al. 2008), *B. micra* could have evolved microhabitat characteristics that allowed it to better exploit *tsingy*-dominated niches. One alternative hypothesis is that *B. micra* rafted over to Nosy Hara after *tsingy* development and either colonized the southern valley via eastern slope forest and *tsingy* wall breaks that connect the

eastern coast to the interior valley, or across the beach and mangrove system on the western coast. Townsend et al. (2009) found that the watershed-dispersal theory after Wilmé et al. (2006) and Pliocene-Pleistocene glacial fluctuations likely had minimal effect on the divergence of *Brookesia*, as both events are chronologically much more recent than basal *Brookesia* divergence 63-81 MYA and *B. micra* divergence 22.5 MYA. Therefore, any climactic shifts post 22.5 MYA appear only to explain Nosy Hara colonization and not divergence. *B. micra* ancestor could evolved with these climactic changes without further divergence or speciation, but further phylogenetic and paired geological analysis of Nosy Hara formation are necessary to confirm this. Phylogenetic niche conservatism predicts that sister taxa of *B. micra* share similar niches (Wiens et al. 2010). *B. desperata, B. confidens* and *B. tristis* are from dry deciduous forest habitats in Forêt d'Ambre, Ankarana and Montagne des Français, respectively, three *tsingy*-dominated habitats (Glaw et al. 2012). Given the initial habitat and microhabitat descriptions presented in Glaw et al. (2012), it is difficult to determine whether the more *tsingy*-associated *B. micra* shares niches with these sister taxa and requires further study. However, given that *Brookesia* itself as a genus is almost completely associated with tropical forest and litter (Townsend et al. 2009), it is possible that the *tsingy*-associated *B. micra* or possibly the dry deciduous forest *B. minima* group A clade (Glaw et al. 2012) support phylogenetic niche conservatism. In addition, *B. peramata* (Ankarana) appears to be an example of parallel evolution with *B. minima* group A clade (dry deciduous species) or *B. micra* in that it too has colonized dry deciduous *tsingy* habitat, but further phylogenetic and habitat studies must be undertaken to confirm this.

Conclusion

This study on *B. micra* has confirmed many behavioral traits unique to *Brookesia* and has established an affinity to *tsingy* outcroppings in *B. micra*. In addition, this study has proven that despite its cryptic nature, *B. micra* is locally abundant and has a wider habitat preference and distribution that previously believed. It is entirely possible that the range of *B. micra* could extend to the surrounding islets in the Nosy Hara archipelago and the mainland, and that the range of *B. micra* could extend to other parts of Nosy Hara. Future studies should examine in the north valleys of the island and in the Nosy Hara environs, but it should be noted that accessibility could be an issue. Due to the low patchiness, high density, flexible habitat preferences, a high estimated abundance (70,000-95,000), and the author agrees with the IUCN rating of Near Threatened for *B. micra* (Glaw and Jenkins 2014). Additional future research should examine sister taxon niche preferences and evolution as well as the geological history of Nosy Hara and surrounding landforms.

Conservation Recommendations

While *B. micra* appears to have a stable population size and habitat in a well-protected park, threats exist for the species. The concrete stairwell built by previous MNP administrations is laid right on top of the only temporary stream on the island for a large percentage of its length. The stairwell was built before the description of *B. micra* and the two exclusively endemic frog species on the island *Stumpffia hara* and *Mantella cf. viridis*. While the streambed itself does not appear to be critical to *B. micra*, it still buried a good amount of habitat and now speeds up the flow of water, promoting erosion. The stairwell appears to fulfill little purpose, as it was built to facilitate tourism to a cave and a viewpoint, but the terrain after the second stairwell is more difficult than the terrain which the stairwell was built over. Tourists who would have found the terrain now under the stairs difficult will find the upper portions of the trail impassable. In addition, the frogs exclusively endemic to Nosy Hara *Stumpffia hara* and

Mantella cf. viridis appear to be only associated with the streambed and stagnant pools used for breeding, and were most likely extremely impacted by the stairwell (Crottini et al. 2012, Köhler et al. 2010). Therefore, it is the recommendation of the researcher that 1) trail maintenance is minimized as much as possible on the *Brookesia micra* trail and 2) the concrete stairway is broken up with as minimal impact as possible to facilitate pool renewal and limit erosion. The researcher also observed invasive rats (*Rattus rattus*), which mainly stayed on the southern beach but were also observed 250m along the southern valley trail. In addition, the researcher urges tourists and future researchers to plan visits and future research with as little bushwhacking off-trail as possible. Even with the high density and large estimated population size, *B. micra* does inhabit an extremely small area, and any disruptions on even a small scale could dramatically reduce available habitat and population size.

Adult male *B. micra* with attached snail.

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Appendix A. Photos of each habitat quadrats for each 50m along southern valley trail. Abundance listed is from day and night transects and opportunistic searching, not each quadrat.

Top: 50m (0 *B. micra*) Bottom: 100m (0 *B. micra*)

Top: 150m (1 *B.* micra) Bottom: 200m (5 *B. micra*)

Top: 250m (10 *B.* micra) Bottom: 300m (5 *B. micra*)

Top: 350m (3 *B.* micra) Bottom: 400m (4 *B. micra*)

Top: 450m (14 *B.* micra) Bottom: 500m (16 *B. micra*)

Top: 550m (7 *B.* micra) Bottom: 600m (10 *B. micra*)

Top: 650m (8 *B.* micra) Bottom: 700m (9 *B. micra*)

Area around 700m quadrat showing lack of litter and roughness of *tsingy* fall field.